The impact of prenatal stress on basal nociception and evoked responses to tail-docking and inflammatory challenge in juvenile pigs

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Abstract

The consequences of tail-docking (at 2- 4 days) and prenatal stress (maternal social stress during the 2nd third of pregnancy) on baseline nociceptive thresholds and responses to acute inflammatory challenge were investigated in juvenile pigs in two studies. Nociceptive thresholds were assessed on the tail root and on the hind foot using noxious mechanical and cold stimulation before and after acute inflammatory challenge by intradermal injection of 30 µg capsaicin, (study 1) or 3% carrageenan (study 2) into the tail root. Four groups of 8 (study 1, n = 14-16 pigs/treatment) or 5 (study 2, n=6 pigs/treatment/sex) week-old pigs were exposed to the main factors: maternal stress and treatment (docked vs. intact tails). In study 1, tail docking did not significantly alter thresholds to noxious mechanical stimulation, while prenatally stressed pigs had significantly higher baseline thresholds to noxious mechanical stimulation on the tail root and on the hind foot than unstressed pigs, whether tail-docked or intact. Capsaicin injection induced localised mechanical allodynia around the tail root in all treatment groups, but had no effect on noxious plantar mechanical responses; however prenatally stressed offspring exhibited significantly attenuated response thresholds to capsaicin compared to controls. In study 2 tail docking did not alter thresholds to either mechanical or noxious cold stimulation. Baseline response durations to noxious cold stimulation of the tail root were significantly shorter in both sexes of prenatally stressed pigs, while male but not female prenatally stressed pigs exhibited significantly higher baseline thresholds to mechanical stimulation than controls, although results in female pigs tended toward significance ($p = 0.07$). Carrageenan injection into the tail root induced localised mechanical and cold allodynia in all treatment groups, effects that were attenuated in prenatally stressed pigs. Collectively, these findings indicate that prenatal stress can induce long-term alterations in nociceptive responses, manifest as a reduced sensitivity to noxious mechanical and cold stimulation and evoked inflammatory allodynia. Neonatal tail-docking does not lead to long-term alterations in nociception in pigs.

Keywords: pig, prenatal stress, allodynia, hyperalgesia, tail-docking, nociceptive processing.

1. **Introduction**

Early life events can result in long-term changes ('programming') of biological functions with significant impacts on health and quality of life. It has long been recognised that exposure of the mother to stress during pregnancy can program the hypothalamic-pituitary-axis (HPA) in the developing foetus [9,38,50,51,52,68,69,71]. The reported effects of prenatal stress[†] on HPA-related responses in human and rodent offspring are diverse and sometimes conflicting [61,65,66,67,68,69,71,72]. Studies on pigs have used pharmacological models of prenatal stress such as the administration of exogenous adrenocorticotrophic hormone (ACTH) or glucocorticoids during pregnancy [11,25], or restraint stress [27,42]. In a recent study by Jarvis and colleagues [26], the effects of prenatal stress on offspring behaviour were investigated in pigs by subjecting primiparous female pigs to social mixing during the second third of pregnancy (a commercially relevant approach to inducing prenatal stress). This work provided clear evidence that prenatal social stress resulted in offspring with increased HPA reactivity as measured by elevated salivary cortisol and corticotrophin-releasing hormone (CRH) mRNA expression in the paraventricular nucleus (PVN) and amygdala [26].

Studies in human infants and rodents have demonstrated that prenatal stress can alter sensitivity to an inflammatory pain challenge; however the reported effects are equivocal [44]. In some studies prenatal stress decreased pain sensitivity [55,56,58], while in others pain sensitivity was enhanced [11,12,13,59]. It has also been reported in humans that the experience of intense pain shortly after birth (neonatal) can in itself induce re-programming of an individual's sensitivity to painful stimuli [3,4,24,32,65]. In production systems, neonatal pigs are exposed to a number of early postnatal painful insults which may include tail docking, and consequently the potential for programming of pigs to induce altered sensitivity to pain later in life exists. Tail docking neonatal pigs, a husbandry procedure practised on many farms across Europe, remains a controversial issue. Behavioural and physiological studies suggest that pigs experience some degree of acute distress after tail docking [41,46,57]. It has also been proposed that tail docking may produce long term sensitivity in the tail stump [54], however firm evidence to support this notion is lacking. The studies reported in this paper were designed to investigate i) the effect of neonatal pain, tail-docking, on nociceptive responses in juvenile pigs and ii) the impact of prenatal stress on long term basal nociceptive responses and on evoked responses to acute inflammatory pain. It was hypothesised that tail-docking would induce chronic allodynia and / or hyperalgesia and enhance inflammatory hyperalgesia induced by an acute inflammatory agent, and that prenatal stress would enhance both effects.

[1] Prenatal stress is defined as stress experienced by the pregnant mother which affects the development of the offspring (Braastad, 1998) [9].

2. Methods

The study was carried out under a UK Home Office project licence and personal licences in accordance with the UK Animals (Scientific Procedures) Act 1986. The experimental protocol (including power calculations) was reviewed and approved by the Animal Experiments Committees of the Scottish Agricultural College and the University of Glasgow.

2.1. Animals

Large White × Landrace primiparous females (gilts) were used in the studies. Thirty six gilts were used in study 1 and 18 gilts were used in study 2. Sixty-one eight week-old female offspring were used from the 1st batch of gilts (study 1) for nociceptive testing and 48 five week-old male and female offspring used for nociceptive testing from the 2nd batch of gilts.

2.2. Maternal procedures

Gilts were kept in groups of six in an environment-controlled gestation house. Gestation pens consisted of 6 individual feeder spaces (1.8 × 0.5m) a dunging passageway (3.6 × 1.9m) and a bedding area of straw covered concrete (2.6 × 2.5m). Gilts were checked daily for signs of oestrus and artificially inseminated at approximately 8 months of age. Individual oestrus cycles were not artificially synchronised. Stress treatment gilts were exposed to social mixing during the second third of pregnancy. This involved moving the gilts into a new pen with three older multiparous sows on two different occasions (2 different groups of multiparous sow) between 39–45 and 59–65 days of pregnancy. This procedure causes profound social defeat [26, 46], and as a consequence is highly stressful.

Three groups of six gilts were allocated to the prenatal stress treatment, with the remaining three groups acting as controls. On the morning of mixing, each group of six gilts was weighed and split into 2 sub groups of three. The first sub-group was moved together to the home pen of 3 older sows. The dimensions of the sow pens were the same as the gilt home pens. During the time when the gilts were being moved for mixing, the resident sows were restrained in the feeders. Once the gilts were in the pen, the sows were released from the feeders. The time taken for splitting the six gilts to releasing the sows took approximately 2 minutes. This process was repeated for all mixed gilts. The mixing of both sets of gilts was timed to happen as close to 10:00 h on the mix day as possible (all mixed occurred between 09:55 and 10:05 h). The gilts then remained in the sub-group for one week before being returned to their home pen. The process was replicated for the second mix: gilts were kept in their same sub-group of three but were exposed to different sows. Gilt age and weights were balanced across treatment groups: average age at farrowing was (517.5 ± 10.4 SE) days and there was no difference in these variables between treatments. Successive groups of 6 gilts were served at approximately monthly intervals. The second batch of gilts was treated in according to the same mixing protocol. Control gilts remained in their home pen undisturbed throughout their gestation period.

Gilts were fed commercial diets appropriate to their stage of gestation. Artificial lighting was provided between 08:00 and 16:00 h. For the farrowing stage, gilts were moved to an individual commercial farrowing crate (2.25 x 0.5 x 1.05m) 5 days prior to expected time of parturition. Twelve farrowing pens, 6 in two adjacent rooms, were located in an environmentally controlled building next to the gestation pen building.

The piglets were able to move around the gilt and had permanent access to a sheltered creep area providing additional heat located behind the gilt feeding trough. No supplemental feed was given to the piglets, although they were introduced to a small amount of feed prior to weaning. The piglets were ear-tagged and weighed on day one of life and received an oral dose of iron supplementation. The piglets were not subjected to castration, teeth clipping or teeth grinding. Average litter size did not differ between the mixed and control gilts (mixed: 12.1 ± 0.8, control: 11.5 ± 1.0). There was no cross-fostering.

2.3. Offspring procedures

2.3.1. Tail docking/sham docking

Piglets were tail docked 2-4 days after birth. Each piglet was manually removed from the litter one at a time and returned to the farrowing pen before the next piglet was taken. Whole litters were either docked or left intact, there was no mixing of treatments (docking/sham docked) within litters. All docking was carried out in the middle of the morning period. No attempt was made to standardise the time from last suckling bout before docking, although piglets were allowed to complete suckling before docking commenced. There was no difference in the average day (3 ± 1 day) of docking between control and prenatally stressed litters.

Approximately one half of the tail was removed using sterile surgical cutters. The piglet was held by one person while another person removed a portion of the tail. Typically the tail was cut around the 5^{th} – 6^{th} coccygeal vertebra. A sample of 20 tails balanced across treatments was evaluated and the average amount of tail removed was 49 ± 2 mm. No analgesia was provided, in accordance with routine farm practice. Sham docked pigs were handled in the same way as the docked pigs without docking.

2.3.2. Weaning

Approximately 28 days (28.3 ± 0.4 SEM) after farrowing, the sow was removed from the farrowing crate. The weaned piglets were kept in this environment for 2-3 days before being transferred to growing pens for the remainder of the experimental period. Days before removal of the sow, the pigs were gradually introduced to solid pelleted food.

2.3.3. Offspring Housing

After weaning, whole litters of pigs were moved to housing containing 12 concrete floored growing pens (2.85m × 1.85m) in a separate building 50 m from the farrowing building in which all experimental procedures were undertaken. The pigs were moved from the farrowing house to the weaner/growing building in batches of 5-6 pigs in a purpose designed animal transporting trolley. The pigs were housed one litter per pen and were provided with deep straw bedding and *ad libitum* access to food and water. The pigs were fed a standard commercial pelleted weaner/grower diet. Lighting was provided between 06:30 and 17:30 h. Pens were cleaned every morning and the straw was replenished daily. The pigs were maintained under these conditions throughout the remainder of the study with minimal disturbance except for routine husbandry. Numbers of animals per pen were reduced to 8 pigs approximately 2 weeks after transfer to the grower accommodation to standardised pen stocking densities. Pigs were randomly picked for removal, unless they appeared under weight.*2.3.4. Nociceptive testing*

Assessment of mechanical nociceptive thresholds was achieved using 1) mechanical stimulation of the tail root using von Frey filaments [14,35,36,48], and 2) mechanical stimulation of the hind foot using a purpose designed plantar stimulator [49]. Assessment of noxious cold thresholds on the tail root was achieved using the ice-cold acetone drop test [15.]

2.3.4.1. Habituation

In study 1, pairs of 8 week old female pigs from each litter were habituated to the investigators, test environment and procedures for a 1-2 week period before the start of nociceptive threshold testing [7]. The time taken for habituation varied between pairs of pigs but usually took between 5-8 days. Habituation involved separating the two test pigs from the litter and walking them from the home pen no more that 5m to a test pen, a large modified pen, located at the end of the pen block containing a straw covered test area with a straw bale (1.85m x 1.85m) and a grilled partition with a gate leading to the plantar stimulator platform (Figure 1A). Once in the test pen the pigs were allowed a short period of time (approximately 5-10 minutes) to settle into a calm behavioural state (standing, head down, snout rooting in substrate unconcerned by human presence or activity in the pen). Habituation sessions also included allowing the pigs to walk-on and investigate the foot stimulator platform (Figure 1B).

The pigs in study 2 were habituated to the investigators and test procedures in their home pen. The 4 allocated test pigs were isolated from the rest of the litter in a section of the pen, with the aid of 2 straw bales (1.0 × 1.0m). Again, the pigs were accorded a short period of time to settle into a quiet, calm demeanour prior to testing. A calm state was induced with the aid a 5% sucrose solution sprayed onto the substrate prior to testing to focus the pigs' attention. Habituation was carried out twice a day at approximately 09:30 and 14:00 h in both studies.

2.3.4.2. Mechanical force stimulation of the tail root

Mechanical thresholds were measured using a calibrated series of nylon filaments (Touch-Test Sensory Evaluator, North Coast Medical, Inc., California, USA). Threshold responses were determined using the 'staircase' force application method described by Tabo and colleagues [59] in rodents. Briefly, the force imparted by bending the filament against the skin was sustained for a period of 2-3 seconds (Figure 1A). This process was repeated 3 times for each filament in ascending force order until a behavioural response was evoked. Upon producing a response the filament was re-applied a minimum of 2 times to confirm a positive result at that force magnitude. A filament of two force loads was then re-applied and the process of applying the monofilaments in ascending force order was repeated until 3 positive threshold responses were obtained. A minimum interval of 30 sec was employed between individual filament applications.

Testing was performed on the dorsal surface of the skin on either the left or right hand side of the tail root on an area of approximately 2 cm^2. This test area was chosen as it was common to both intact and tail-docked pigs, and was considered to be close enough in proximity to the injured tail end to be affected by localized modifications in nociceptive sensitivity in the pigs' tail after docking. Preliminary studies [48] showed that before the administration of an acute inflammatory challenge, the pigs exhibited 3 key response behaviours to mechanical stimulation, the most prominent and consistent response being a rump tuck/flinch. (Table 1). The pigs also exhibited a novel response to mechanical force stimulation after acute inflammatory

challenge in the form of vigorous tail flicking, a response previously reported in pigs in response to noxious electrical and thermal stimulation [16,45].

2.3.4.3. Noxious mechanical stimulation of the hind foot.

Threshold responses to noxious mechanical stimulation of the hind foot were assessed using a purpose designed plantar stimulator [49]. Briefly, over a period of approximately one week the pigs were habituated to walking onto a perforated platform with a feed/drink dispenser at one end [49]. A mechanical force stimulator and force measurement device was positioned beneath the platform and was guided by an operator with the aid of an incorporated miniature camera and video monitor to a suitable hole beneath the pig's hind foot. A microchip-controlled stimulator head advanced a stainless steel probe (2 mm diameter) mounted on a force transducer via a stepping motor linear actuator [49]. The stimulus probe was driven upwards into the plantar surface of the pig's hind foot and the force imparted was measured over the duration of stimulation. The probe was advanced until the pig withdrew its foot from the stimulus. This point was detected by the measured force dropping more than approximately 0.6 N below its maximum recorded value, upon which the probe was fully retracted beneath the platform. Three measurements of threshold force (peak force [N] before foot withdrawal) were obtained in each test session. A minimum interval of 30 s was employed between stimulus applications to minimize the possibility of sensitizing the foot pads. The stimulator was pre-programmed with a 15 N cut-off to prevent injury to the pig's foot. All acquired data was automatically output to a data file for further analysis in commonly used PC data processing software.

Plantar pad testing was not carried out on 5 week old pigs due to practical limitations and extended time for effective habituation to the procedure.

2.3.4.4. Noxious cold stimulation of the tail root

Responses to noxious cold stimulation were measured in 5 week old pigs (study 2) using the acetone drop test [15]. Ice-cold acetone (ca.0-2°C; 50 µl) was dispensed on the tail base using a positive displacement pipette (Microman, Gilson Scientific, Luton, UK). This procedure was repeated three times to obtain average response duration. A minimum interval of 1 minute was employed between acetone applications. Tail responses followed a typical pattern starting with an initial rump tuck/flinch followed by rapid vigorous tail flicking or clamping. The duration of all response behaviours (i.e. from the start of initial rump tuck to end of tail flicking or clamping) was determined by retrospective analysis of digital video recordings taken at the time of the testing using high resolution IR colour CCTV cameras (LIT40ESHQ, ezCCTV.com Ltd, Letchworth, UK) linked to Geovision® surveillance system and video analysis software (GV-1240, ezCCTV.com Ltd, Letchworth, UK). During analysis, the observer was blinded to maternal treatment but was aware of tail treatment and gender.

2.3.5. Induction of peripheral inflammation

Prior to injection, the test pigs were moved into a corridor (1m) adjacent to the home pen. The pigs were restrained (sternally recumbent over a straw bale) by staff experienced in pig handling, and received an intradermal injection of 30 µg capsaicin (0.2 ml) dissolved in 10% Tween 80 in sterile phosphate buffered

saline (study 1) or 3% carrageenan in phosphate buffered saline, 0.1ml, (study 2) into an area adjacent to the tail base, administered using a sterile 25 gauge insulin needle. (Dunlops Veterinary Supplies, Dumfries, UK). Injection order was randomly determined beforehand. After injection, the pigs were immediately returned to their home pen. None of the pigs injected exhibited overt signs of distress or pain and quickly settled back into their routine once returned to the home pen.

All chemicals used in these studies were obtained from Sigma-Aldrich Co., Poole, UK. A preliminary description of the inflammatory models used in this study has been reported previously [48]. Visual signs of inflammation (redness and swelling) occurred within a few minutes of injection with capsaicin, but took longer (>1h) to develop following carrageenan injection. In previous studies [48], the observed area of inflammation lasted approximately 1h, although elevated sensitivity to mechanical stimulation persisted beyond 2h and appeared to resolve by 4h. In the same study carrageenan injection produced an obvious area of inflammation that was still visible in some cases 48h after injection.

2.4 Experimental Design

2.4.1 Study 1

Study 1 was based on a 2x2 factorial design with prenatal stress and tail docking as main factors. Sixty-one 8-week-old female offspring were tested from 31 different litters) from 4 maternal/offspring treatment groups: control (not mixed)/ tail intact [CI], control tail-docked [CD], stressed (mixed) tail intact [MI], stressed tail docked [MD]. Nociceptive testing was performed on pigs (52-58 days of age). On day 2 of testing, pigs were subjected to an acute inflammatory challenge in the form of an intradermal injection of capsaicin, into the tail root. Response thresholds to mechanical force stimulation of the tail root (von Frey filaments) and hind foot (plantar stimulator) were recorded 24 hours and 30 minutes before, and 30, 60, 120 and 240 minutes after capsaicin injection [48].

2.4.2 Study 2

Study 2 was based on a 2x2 factorial design with prenatal stress and tail docking as main factors. Forty-eight 4-5-wk-old male (24) and female (24) pigs were obtained from 12 different litters from 4 maternal/offspring treatment groups described in study 1. Nociceptive testing was performed on pigs aged 29-36 days. On day 2 of testing, the pigs were subjected to an acute inflammatory challenge in the form of an intradermal injection of carrageenan, into the tail root. Response thresholds to mechanical stimulation (von Frey filaments) and response durations to noxious cold thermal stimulation (ice-cold acetone drop) at the tail base were recorded 24 hours before, and 1, 6, 24 and 48 hours after carrageenan injection.

2.5. Statistical analysis

Prior to statistical analyses, response variables (von Frey filament threshold response force and peak force before foot withdrawal were checked for normality and equal variances (box-plot, data not shown). Response thresholds measured by von Frey filaments had been $\log_{10}$ transformed to normalise the data. Von Frey mechanical force thresholds were measured in milli-newtons (mN) and presented as log mean ± 1 standard deviation (Log mN). Foot withdrawal peak force measurements were normally distributed. In Study

2, some nociceptive threshold responses were attained using the lowest filament available (0.08 mN) -due to younger age of pigs) producing censored data (30/240 values). Kaplan-Meier survival analysis [28] was performed to generate probability curves for the censored data. Predicted values based on probability curves were then randomly allocated to censored data values.

All data were analysed using General Linear Model (GLM) repeated measures analysis of variance and Bonferroni's pair-wise comparison test of mean differences. GILT (control, mixed) and TAIL (intact, docked) and their interaction were included as fixed factors in the model, with TIME as a 3rd fixed factor and SUBJECT as a random factor nested within groups. Statistical analyses were carried out using Minitab version.15. Calculations of area under the curve (AUC) for time series data were calculated using the trapezoid method and analysed using GLM analysis of variance with Bonferroni's pair-wise comparison test of mean differences. This approach was also used to compare pre-injection (baseline) differences between treatments.

3. Results

3.1. Study 1

Pigs that were prenatally stressed (MI and MD) had significantly higher (p<0.05) baseline response thresholds to mechanical stimulation of the tail root than control pigs, either tail docked (CD) or intact (CI) (Figure 2). Baseline mechanical thresholds were 27% and 20% higher in the MI and MD pigs than in there respective control groups. Collectively, prenatally stress offspring exhibited significantly higher (p<0.05) foot withdrawal thresholds following noxious mechanical plantar stimulation (Figure 3) than the two non-stressed treatment groups (MI vs. CI 14%, MD vs. CD 10 %).

Capsaicin produced a significant reduction (p<0.05) in mechanical thresholds 30 minutes after injection in all groups (Figure 4A) compared with pre-injection thresholds, that was sustained up to 4h (*p<0.05*). Prenatally stressed pigs exhibited significantly attenuated (p<0.05) thresholds after acute inflammatory challenge with capsaicin, whether intact or tail docked. Integrated response force thresholds (vs. time $AUC_{0\text{-}240\ min}$) shown in Figure 4B were significantly higher (p<0.05) in both prenatally stressed groups than in the unmixed control groups following capsaicin challenge (MI vs. CI 125%, MD vs. CD 73%). Plantar mechanical thresholds did not alter significantly over time in response to capsaicin injection into the tail root, however there were significant differences (p<0.05) between the prenatally stressed pigs and their respective controls 30, 120 and 240 min after inflammatory challenge (Figure 5A). Integrated plantar mechanical thresholds ($AUC_{0\text{-}240}$ min) were significantly higher (p<0.05) in prenatally stressed and tail-docked pigs (MD) than in the CD pigs following acute inflammatory challenge (MD vs. CD 20 %), but not significantly different between MI and CI pigs (MI vs. CI 15% - Figure 5B).

Tail docking had no significant effect on baseline response thresholds to mechanical stimulation around the tail root (Figure 2), plantar surface of the hind foot (Figure 3) nor subsequent responses to capsaicin-induced inflammation either measured at the tail base (Figures 4A and 4B) or plantar surface of the hind foot (Figures 5A and 5B).

3.2. Study 2

Male prenatally stressed pigs had significantly higher (p<0.05) pre-injection (baseline) response thresholds to mechanical force stimulation of the tail root than control pigs, either tail docked (CD) or intact (CI) (Figure 6B). Baseline mechanical thresholds were 48% and 26% higher in the male MI and MD pigs than in their respective control groups. Baseline response thresholds were not significantly different (p= 0.072) in female prenatally stressed and control groups (Figure 6A). Intradermal carrageenan induced a significant reduction ($p<0.05$) in mechanical thresholds in all treatment groups, in both sexes (Figures 7A and 7C), and was greatest 1 and 6 hours post injection. Mechanical thresholds were still significantly lower ($p<0.05$) 24 hours after carrageenan injection in all treatment groups in both sexes, but not at 48 hours post-injection. There was no effect of tail docking in either sex on nociceptive thresholds following inflammatory challenge. Integrated response force thresholds (force vs. time $AUC_{0\text{-}48h;}$ Figures 7B and 7D) were significantly higher (p<0.05) in the male prenatally stressed groups than in the unmixed control groups (MI vs. CI 54%, MD vs. CD 19%) but not significantly in the females (p=0.06) after carrageenan challenge.

Baseline response durations to noxious cold stimulation of the tail root were significantly shorter ($p<0.05$) in duration in the prenatally stressed pigs of both sexes compared with their control counterparts (Figures 8A, 8B), indicating reduced responsiveness. Carrageenan injection into the tail root induced significant increases ($p<0.05$) in nocifensive response durations (tail clamping and flicking) in all treatment groups and in both sexes (Figures 9A and 9C), with maximum effects ($p<0.05$) at 6 h after injection in males and females respectively. Response durations were significantly ($p<0.05$) lower in male pigs from all treatment groups 24 hours after carrageenan injection, and remain significantly lower in female pigs at 48 hours post-injection. Pigs from both prenatally stressed groups (intact and docked) in both sexes exhibited significantly lower ($p<0.05$) integrated response durations following carrageenan-induced inflammation (Figures 9B and 9D), reflecting a reduced nocifensive response to carrageenan induced inflammation compared with control pigs.

There was no significant effect of tail docking on mechanical threshold sensitivity or noxious cold stimulation responses in either maternal treatment group, male or female.

4. Discussion

This work found no evidence that tail-docking induces long term changes in nociceptive sensitivity in pigs and that prenatal stress induces alterations in nociceptive information processing in pigs manifest as an elevation in baseline nociceptive thresholds (hypoalgesia) and attenuation in mechanical force sensitivity and response duration to noxious cold stimulation to acute inflammation.

The absence of an effect of tail docking on nociceptive responses in pigs 5 - 8 weeks after injury is interesting. It is acknowledged that tail docking causes acute pain in pigs [41,43,46,57,64] however, although the long-term effects of docking on tail pain sensitivity until now have not been assessed objectively, much has been written about the potential long term impact on pig welfare. It had been hypothesised that chronic hyperalgesia occurring as a consequence of traumatic tissue injury and the potential formation of neuromas at the ends of the cut nerve trunks after tail docking [53] would develop. The results from the studies reported in this paper showed no alterations in mechanical nociceptive thresholds in tail-docked pigs, i.e. no evidence of chronic hyperalgesia. Study 2 was carried out to address the concern that 8 weeks after tail-docking may have been too long to identify an effect. This follow up study confirmed no effect at 4- 5 weeks of age. Amputation of the mouse tail tip has been shown to cause long term thermal and mechanical

hyperalgesia in the remaining part of the tail [72], however in contrast to this pig study where tail tip amputation was carried out 3 days after birth (neonatal), tail tip amputation in the mouse study was performed in 4-6 week-old mice. This is may account for the different results.

In humans strong, sustained noxious stimulation in neonates can produce long term alterations in pain sensitivity; however the consequences on future pain responses depend on the specific conditions of the neonatal noxious insult [33]. The lack of a long-term effect of tail-docking on nociception indicates that the impact of docking is limited, probably to the time taken for the tail stump to heal, and that the stimulus was not sufficiently intense/long-lasting to induce long-term changes. It is recognised that by assessing responses to mechanical stimulation at the tail base and not directly at/on the site of tissue damage (tail tip), a localised effect may have been missed, however the biological significance of such an effect would be questionable. Innervation of the pig tail is via two dorsal and two ventral coccygeal nerve trunks supplying the sensory and motor fibres to the muscles, sensory hairs and glands of the tail. Proximally, the dorsal branches of the coccygeal nerves join the fourth sacral nerve to form a dorsal caudal plexus and as such share common inputs to the spinal cord with nerves that innervate the hind limbs and pudendal region. Thus if tail docking induced central sensitization, these shared sensory inputs could induce secondary hyperalgesia at sites distal to the site of tail injury (e.g. hind foot). This was not shown, thus indicating no long term impact of tail-docking in pigs on nociception.

Previous studies on pigs [26, 45] indicated that experience of a social stressor during pregnancy produced long term alterations in offspring behaviours and responses; however this is the first report of prenatal stress induced effects on basal nociceptive responses and on evoked nociception. Pigs from mothers stressed during the second third of pregnancy were less responsive to noxious stimulation, with higher basal response thresholds and reduced integrated responses to inflammatory challenges. These effects are consistent with findings from several studies in rodents where nociceptive responses were attenuated in the offspring from pregnant females subjected to restraint stress during mid and late pregnancy [29,54,55]. Pre-natal stress appears to programme long-term changes in the developing somatosensory system of the pigs to produce alterations in both nociceptive and tactile sensitivity. The results in study 2 showed a clear effect of prenatal stress on cold nociception in both male and female pigs, and on mechanical nociception in male pigs. The lack of significant effect on mechanical nociception in female pigs may have been a consequence of the smaller group sizes (6 pigs per group) compared with the group sizes in study 1 (14 – 16 per group), but there was a clear tendency toward significance ($p = 0.07$). Both studies indicated that pre-natal stress did not alter the lack of long term effect of tail-docking on pigs.

There is some debate about whether von Frey filaments assess noxious sensory input. The force range of filaments used in these studies ranged from 0.08 to 2941.18 mN (-1.11 to 3.47 log mN). Lynn and colleagues [36] reported that force thresholds above 10 mN (1 Log mN) stimulated both mechanical and polymodal c-fibre afferents in juvenile pig skin. Baseline responses across all treatments in the 8 week old pigs (study 1) occurred around 1.89-2.41 Log mN (78-255 mN) indicating stimulation of c-fibres. In the 5 wk old-pigs (study 2) baseline responses to von Frey filament stimulation were generally less than 10mN, the activation thresholds described by Lynn and colleagues. However the electrophysiological studies were undertaken in anaesthetised pigs and the impact of general anaesthesia on the data is unknown, which may have altered the the firing thresholds of c-fibres. [36].

The capsaicin [30,52,62] and carrageenan [28] models of inflammatory pain has been shown to induce mechanical and cold allodynia [1,18] and hyperalgesia [1, 30]. The observed attenuation of mechanical threshold and decrease in response durations to noxious cold stimulation local to the site of injection in the prenatally stressed pigs is consistent, in part, with some reports in rodents [29,54] which reported that offspring from pregnant mothers in mid to late pregnancy repeatedly subjected to periods of restraint (a recognised stressor) exhibited increased tail or paw withdrawal latencies to the hot plate test, but are not consistent with others where the effects of prenatal stress increased response latencies in formalin-induced pain in rats [12,13]. An explanation for the divergent effects may lie in the intensity and chronicity of the postnatal nociceptive challenges [12]. Nociceptive responses in rodents and humans to persistent inflammatory irritants such as formalin and Complete Freunds Adjuvant (CFA) differ markedly from those to acute nociceptive stimuli such as capsaicin and carrageenan [6,28,30,46,52,62]. The short term nociceptive effects of intradermal capsaicin (ca. 2 hours) and carrageenan (<24h) observed in these studies are more in line with those reported by Sternberg and colleagues [55] using the hot-plate test and support the proposition that the persistency and severity of the nociceptive challenge used may be important in determining response outcomes.

Jarvis and colleagues [26] reported that the offspring of prenatally stressed primiparous gilts exhibited enhanced HPA responses and greater anxiety-type behaviour later in life, determined from measures of salivary cortisol and CRH mRNA expression in the PVN and amygdala. It was proposed that prenatal stress resulted in an over reactive emotional/stress phenotype. It is therefore considered that the attenuation in nociceptive responses (both basal and evoked) observed in the present study are a consequence of the effects of enhanced HPA reactivity in the prenatally stressed offspring and as such reflect a form of stress-induced analgesia [2,29,54,55,69].

The impact of basal hypoalgesia and reduced responsiveness to inflammatory pain in the prenatal stress phenotype on pig welfare is not clear. It has been postulated that many of the effects of maternal stress on offspring have an adaptive basis [8, 9, 23] and that maternal stress “programming” may in the short term match the offspring to predicted post natal environmental conditions, that is to say a more reactive phenotype may be more suited to a risky/unpredictable environment [39]. However while this may confer certain advantages in survival terms, it is now recognised that the long-term consequences of prenatal programming offspring with over-reactive HPA responses may lead to the premature onset of detrimental physiological and psychological health issues later in life in humans [5,10,19,20,21,22,38] and other mammalian species [17,32,37,,61,65,66,67]. The potential impact of pre-natal stress on the prevalence of disease in pigs is not known.

5. Conclusions

The data reported in these studies indicate that tail-docking neonatal pigs does not induce long-term alterations in nociceptive responses, thus there is no evidence of chronic hyperalgesia,, and that prenatal stress (*in utero*) reduces responses to acute pain stimuli and acute inflammatory induced nociception in later life.

Conflict of interest

1 The authors have no conflict of interest
2
3 **Acknowledgments**
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8

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Behaviour	Evoked behaviour (%)	
	Pre-injection	Post-injection*
Stimulus avoidance/move away	2.2	0.0
Rump tuck/flinch	86.7	0.0
Tail flick (single, directed)	11.1	8.7
Tail flicks (multiple, multi-directional)	0.0	91.3
Log average response force (mN)	2.70 (500)	0.81(6.5)

Table 1. Threshold behavioural responses (% occurrence) in eight 8 week old female pigs (n=8) evoked by mechanical stimulation of the tail base using von Frey filaments 30 minutes before and after intradermal capsaicin injection into the tail root. Log average response force thresholds (mN) are also presented with back calculated average force values shown in parenthesis.

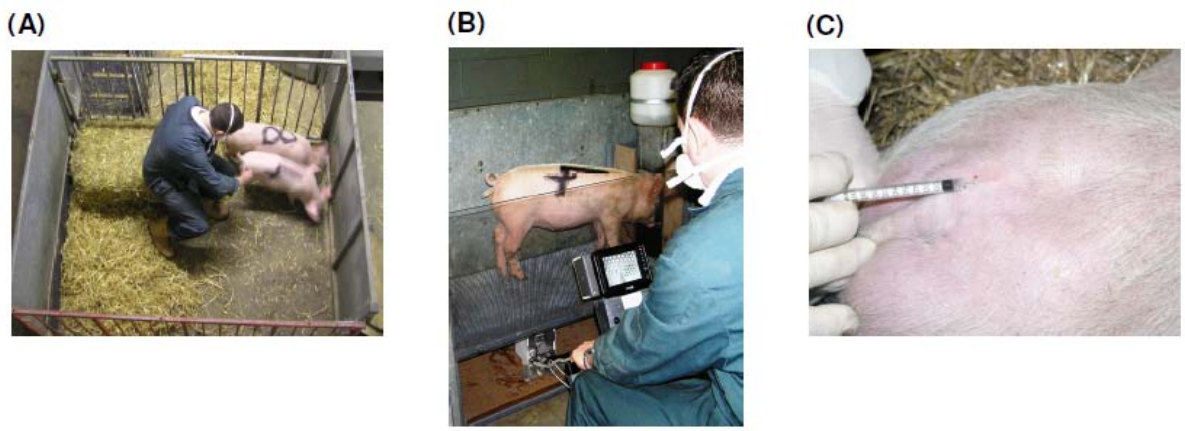

Fig 1. **(A)** Pair of habituated 8 week-old female pigs being tested for mechanical threshold sensitivity with von Frey filaments in area around tail root. **(B)** Operator aligning the plantar stimulator device under the hind foot of a pig standing on the test **(C)** Site of injection for acute inflammatory challenge in the dorsal surface of the tail root.

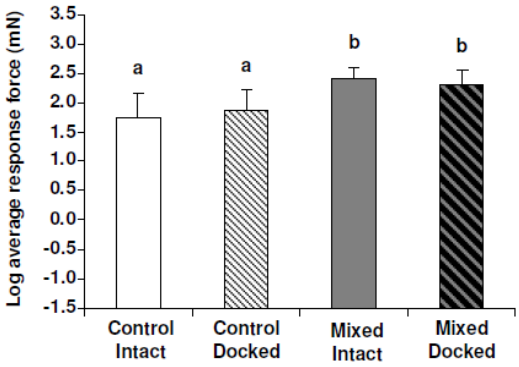

Fig 2. Baseline response thresholds to noxious mechanical stimulation of the tail root in 8 week-old intact or tail docked female pigs from control (unmixed) or stressed (mixed) mothers. Data represent mean ± one standard deviation (SD). Pigs per treatment; control intact (16), control docked (16), mixed intact (14), mixed docked (15). Treatment groups with different letters are significantly different ($p<0.05$). Ordinate axis scale covers the full range of von Frey filaments used in testing (Log mN; min -1.11. max 3.47).

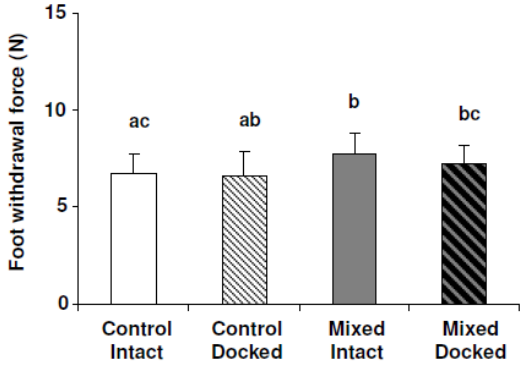

Fig 3. Foot withdrawal thresholds after noxious mechanical stimulation of the hind foot in 8 week-old intact or tail-docked female pigs from control (unmixed) or stressed (mixed) mothers. Data represent mean ± one standard deviation (SD). Pigs per treatment; control intact (16), control docked (16), mixed intact (14), mixed docked (15). Treatment groups with different letters are significantly different ($p<0.05$). Treatments with same letters are not significantly different.

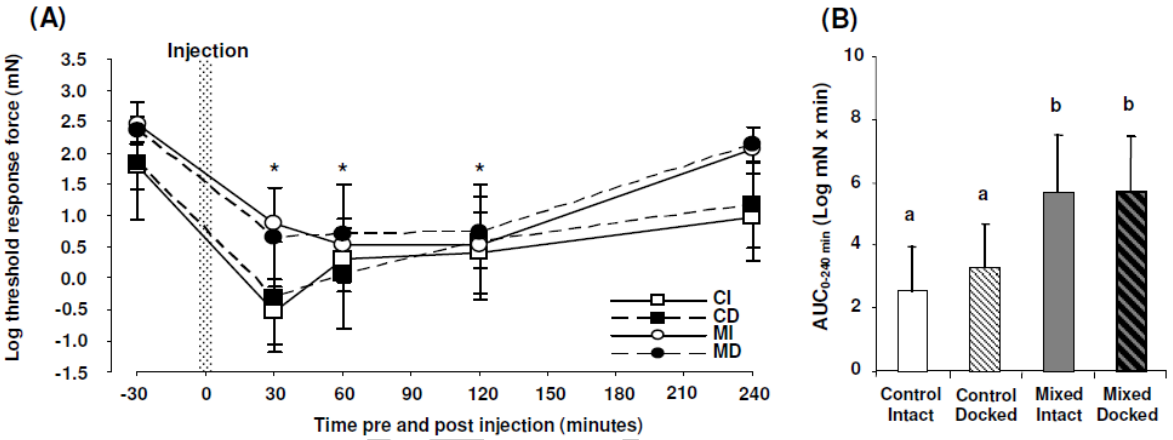

Fig 4. **(A)** Response thresholds to noxious mechanical stimulation before and after acute inflammatory challenge 30 µg intradermal capsaicin injection into tail root of 8 week-old female pigs **(B)** Integrated effect (area under curve [AUC $_{0\text{-}240\ min}$]). Pigs per treatment; control intact (16), control docked (16), mixed intact (14), mixed docked (15). In Fig 4A, asterisks (*) indicate time points at which mechanical thresholds (within treatment) were significantly ($p<0.05$) different from pre-injection values. In Fig 4B, treatment groups with different letters are significantly different ($p<0.05$).

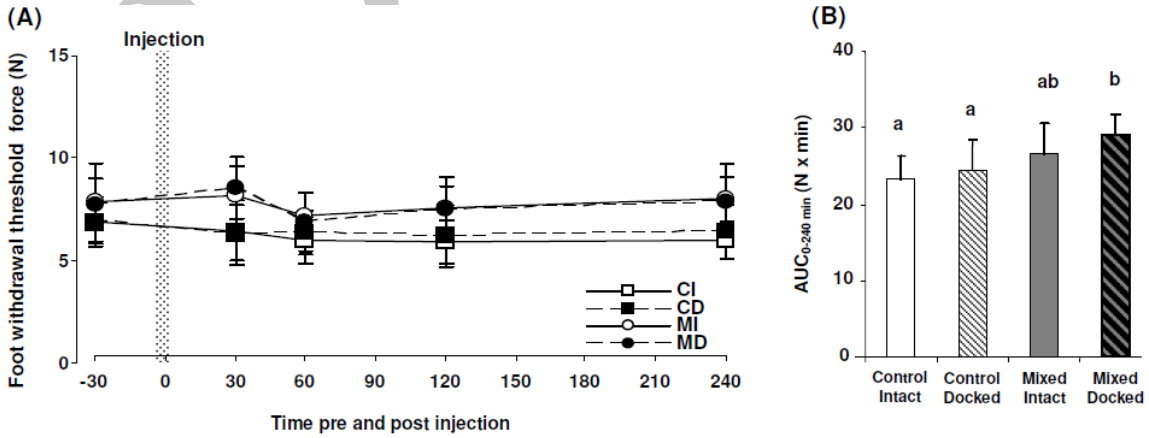

Fig 5. (A) Response thresholds to noxious mechanical stimulation of the plantar surface of the hind foot before and after acute inflammatory challenge 30 µg intradermal capsaicin injection into tail root of 8 week-old female pigs **(B)** Integrated effect (area under curve [AUC $_{0\text{-}240\ min}$]). Data represent mean ± one standard deviation (SD). Pigs per treatment; control intact (16), control docked (16), mixed intact (14), mixed docked (15). In Fig 5B, treatment groups with different letters are significantly different ($p<0.05$).

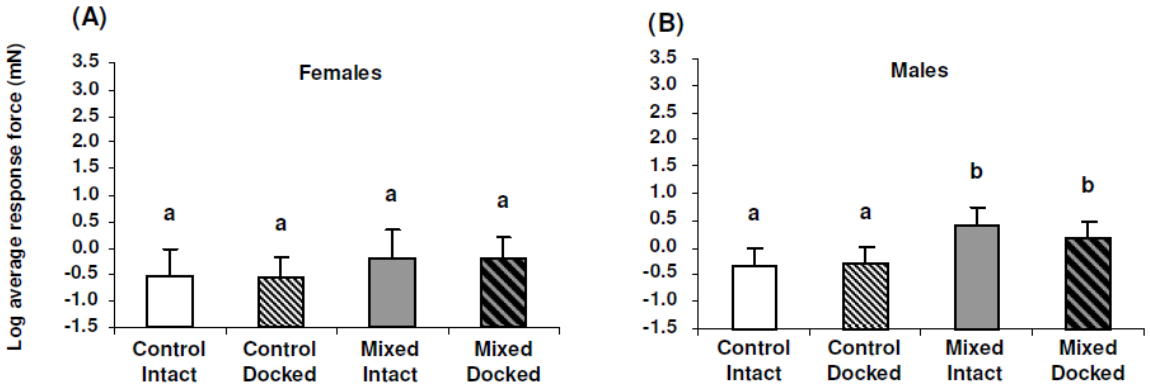

Fig 6. Baseline response thresholds to mechanical stimulation of the tail root in 5 week-old intact or tail-docked female **(A)** and male **(B)** piglets from control (unmixed) or stressed (social mixing during second third of pregnancy) sows. Data represent mean ± one standard deviation (SD), n=6 piglets per treatment. Treatment groups with different letters are significantly different ($p<0.05$).

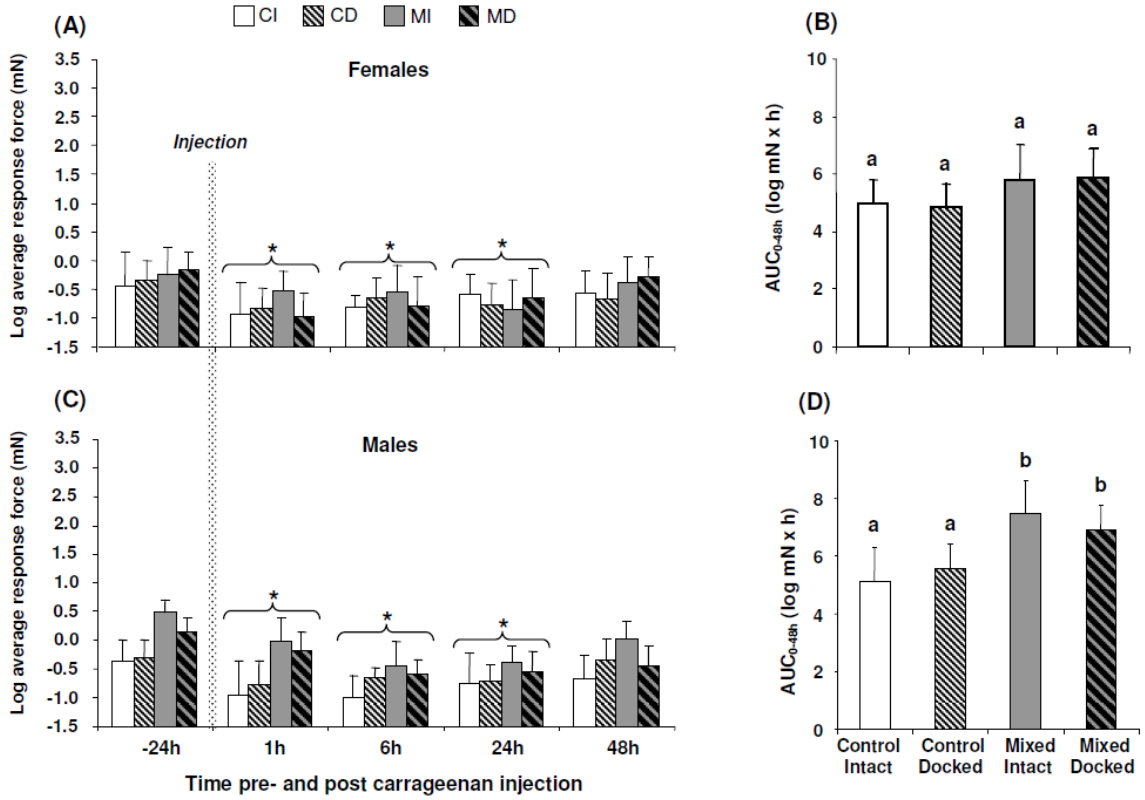

Fig 7. Response thresholds to mechanical stimulation before and after acute inflammatory challenge (intradermal injection of 3% carrageenan solution into dorsal aspect of tail root and integrated effect (area under curve [$AUC_{0\text{-}48h}$]) in 5-week-old female **(A, B)** and male piglets **(C, D).** Data represent mean ± one standard deviation (SD), 6 piglets per treatment/sex. In Fig 7A & C asterisks (*) indicate time points at which threshold responses were significantly different ($p<0.05$) from pre-injection threshold values. In Figs 7B & D, treatment groups with different letters are significantly different ($p<0.05$).

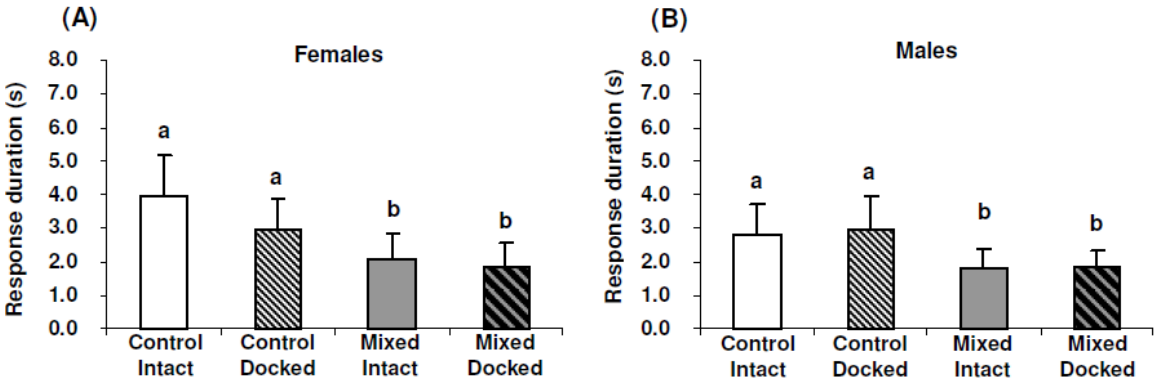

Fig 8. Baseline response durations to noxious cold stimulation (ice-cold acetone, 50µl) of the tail root in 5 week-old intact or tail-docked female (A) and male (B) piglets from control (unmixed) or stressed (social mixing during second third of pregnancy) mothers. Data represent mean ± one standard deviation (SD), n=6 piglets per treatment/sex. Treatment groups with different letters are significantly different ($p<0.05$).

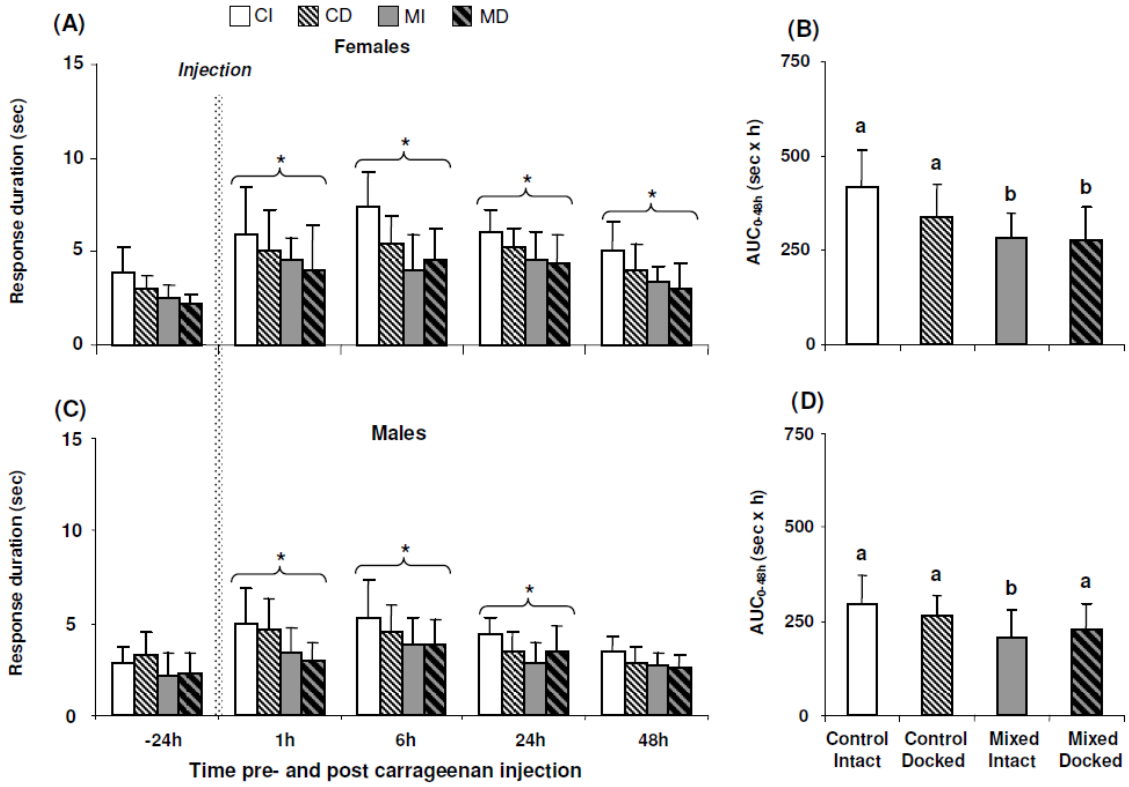

Fig 9. Nocifensive response (tail clamping and flicking) duration following noxious cold stimulation (ice-cold acetone, 50 µl) before and after acute inflammatory challenge (intradermal injection of 3% carrageenan solution into dorsal aspect of tail root), and integrated effect (area under curve [$AUC_{0\text{-}48h}$]) in 5 week old female **(A, B)** and male piglets **(C, D).** Data represent mean ± one standard deviation (SD), 6 piglets per treatment. In Figs 9A&C, asterisks (*) indicate time points at which total response durations were significantly different ($p<0.05$) from pre-injection values. In Figs 9B&D, treatment groups with different letters are significantly different ($p<0.05$).